EFFECT OF SEED SCARIFICATION, RHIZOBIUM INOCULATION AND PHOSPHORUS FERTILIZATION ON ROOT DEVELOPMENT OF BARSEEM AND SOIL COMPOSITION

SOHAIL AHMAD*, GHULAM HABIB*, YOUNAS MUHAMMAD*, IHSANULLAH*, ZEESHAN DURRANI*, UROOBA PERVAIZ** and ALTAF UR RAHMAN*

* Faculty of Animal Husbandry and Veterinary Sciences, NWFP Agricultural University, Peshawar, Pakistan
** Department of Extension Education & Communication, NWFP Agricultural University, Peshawar, Pakistan

ABSTRACT

Barseem (Trifolium alexandrinum L.) is a common rabi grown legume fodder crop that fits well in the farming system at the irrigated lands in Pakistan. Variation in per acre productivity could be attributed to varietals differences and variation in agronomic practices. The current experiment was designed to assess the effect of seed scarification, phosphorus fertilization and rhizobium inoculation on post-harvest soil nitrogen and root development of barseem. The five treatments viz; (A) control, (B) scarified seeds, (C) P-fertilization, (D) Rhizobium inoculation and (E) Rhizobium inoculation + P-fertilization were applied in a Complete Randomized Block Design using three replicates per treatment. Plant height remained the same during the first three and fifth cuts, however, significant (P < 0.05) differences were observed during the fourth cut of barseem. Treatment C and E yielded the maximum height followed by treatment D, A and B. Mean plant height during the successive cuts were 30.1, 47.1, 52.1, 56.7, and 54.3 cm, respectively. Pre-trial soil nitrogen content was 0.12%, which apparently did not change in response to any treatment. Mean soil-N after the last cutting of barseem was 0.068% across the five treatments. Post-harvest phosphorus content in the soil increased in plots, which were fertilized with phosphorus. The low mean phosphorus content in the fodder (0.21 %) may be attributed to the low pre-trial soil phosphorus concentration (71 mg kg⁻¹). Phosphorus application and rhizobium inoculation in isolation or in combination tended to enhance root development i.e., root length, number of nodules and root hairs per plant in the present study but due to large variation between the replicates with in the treatment the effect on root development was statistically insignificant. The average number of nodules per plant (138) in the present study was markedly higher than the maximum number (71) reported in literature. Phosphorus fertilization in isolation or in combination with rhizobium inoculation enhanced phosphorus reserves of the soil and strengthened the root vigour to support more yielding cuts.

Key words: Barseem, Rhizobium inoculation, Phosphorus fertilization, Root nodules, Root length


INTRODUCTION

Among fodders barseem (Trifolium alexandrinum L.) is the most widely grown multi-cut legume crop grown during rabi in Pakistan. It is highly nutritious fodder contains 15.8-26.7 % CP, 1.4-3.0 % ether extract, 14.9-28.3 % crude fiber, 1.4 - 2.58 % calcium and 2.22 - 2.46. Barseem is either grown solely or mixed with other fodder crops like barely, oats or brasica. Its symbiotic activity in increasing soil nitrogen contents has given barseem an importance in crop rotation and is widely grown in the irrigated areas of Pakistan.

Seed cortex of barseem like the other legumes absorb water very slowly at sowing and ultimately germinates after a few days of soaking period in the soil. In some fields where termites and ants are in abundant, utilize the opportunity and stock the seed for food. Seed cortex abrasion is one of the method for quick water absorption and hence an early germination. The process is called scarification (Garza et al. 1987). Phosphorus fertilization causes intensive root growth and enhances nitrogen fixation activity at the nodule level (Pal et al. 1989). Barseem has the quality to provide habitat to soil-borne bacteria (rhizobium) in their root system. The correct strain of rhizobium ensures maximum fixation of atmospheric nitrogen through symbiosis (Kishinvesky et al. 1992). Increase in yield can be obtained through inoculation with rhizobium even in the fields where barseem is grown previously, to obtain residual effect for subsequent crop (Elkin, 1986) with varying performance from strain to strain (Shaheen and Rahmatullah, 1996). In a previous study the effect of the above factors has been studied on DM yield, nitrogen, calcium and phosphorus contents, (7.6, 9.1, 9.3, 9.4 and 9.8 tons ha⁻¹; 3.01, 2.80, 2.90. 2.91 and 2.94 g per
Sohail Ahmad et al. Effect of seed scarification, rhizobium inoculation and phosphorus fertilization root... 370

100 g DM; 3.01, 2.8, 2.9, 2.91, 2.94%; 2.4, 2.4, 2.4, 2.6, 2.3% and 0.20, 0.19, 0.22, 0.21, 0.22%) with treatments control, seed scarification, application of phosphate fertilizer, rhizobium inoculation and the combined effect of phosphate fertilizer and rhizobium inoculation (Ahmad et al. 2001). In the current study the effect of the above treatments were studied on plant height, soil nitrogen, and phosphorus contents, root hairs, root length, number of nodules per plant and root-branching.

MATERIALS AND METHODS

Experimental Design and Treatments

A piece of land 400^2 meter (20m X 20m) was selected and demarcated from the farmland at the Animal Husbandry In-service Training Institute, Peshawar. Selected plot was ploughed two times to a minimum depth of 23 cms followed by application of farmyard manure. After mixing with the manure using third ploughing the land was leveled, divided into 15 equal plots of 4 X 5 meters distributed in three rows. Water channel was furrowed between two rows to insure irrigation of plot individually. Barseem was sowed in standing water. Urea equivalent to 20 kg nitrogen per hectare was applied to individual plot to provide starter nitrogen. The five treatments viz; (A) control, (B) scarified seeds, (C) P-fertilization, (D) Rhizobium inoculation and (E) Rhizobium inoculation + P-fertilization were applied in a Complete Randomized Block Design using three replicates per treatment. Barseem seeds were purchased from the local market and broadcasted in standing water in each plot separately at the recommended rate of 20 kg ha\(^{-1}\) (Shafi et al. 1993). For scarification, rubbing a layer of seeds in between two pieces of sandpaper for about one minute abraded the outer cortex of the seeds. The cultured rhizobium BL2 was obtained from the Soil Science Department of Agricultural Research Institute, Tarnab, and used according to their recommendations. In Treatment C, phosphorus in the form of single super-phosphate fertilizer (P-18%) was applied after irrigation, before sowing at the rate of 100 kg P\(_2\)O\(_5\) ha\(^{-1}\) (equivalent to 1.10 kg per plot) as suggested by Shaheen (1988).

Soil Sampling

Representative soil samples were collected two times during the study with the help of soil sampler. The initial soil samples (about 2 kg) were randomly collected from 5 different sites of the demarcated land. These were pooled and stored. Immediately after collecting the last harvest of barseem, second soil samples were collected. Soil samples were collected from the center of each replicate plot and stored separately. The samples were analyzed for total nitrogen, calcium and phosphorus according to the standard procedure of AOAC (1980).

Counting of Root Nodules and Root Length and Root Hairs

Five plants per plot were randomly uprooted from each plot after 24 hours of collecting the last harvest. The roots along with the adhered soil lumps were soaked in a bucket containing water and the soil was removed gently from the roots. The roots were rinsed with clean water and numbers of nodules on root hairs were counted. Observations on length of plant root, number and length of root hairs were also recorded.

Determination of Plant Height

The plants were harvested manually three inches above the ground. Eight plants per plot were randomly selected from each cut and plant height was determined with the help of measuring tape.

Statistical Analysis

The data of all the parameters were analyzed with the analysis of variance procedure as described by Steel and Torrie (1981). The model included main effects of treatments, cut number and interaction of treatment x cut number. The means were compared for significance of difference with the Duncan Multiple Range Test using SAS (1986).

RESULTS AND DISCUSSION

Plant height averaged 30.1, 47.1, 52.1, 60.0 and 54.3 cms for cuts 1, 2, 3, 4 and 5, respectively (Table-I). The five cuts were obtained at internal of 100, 23, 22, 23 and 26 days and the DM content in the fodder correspond to 12.9, 10.1, 11.1, 14.2, and 20.5 % respectively. Plant height remained unaffected by either of the treatment during the first three and last (5\(^{th}\)) cuts but significant difference was observed in the height during the fourth cut. Treatment
C and E gave the maximum height followed by D, A and B. Plant height was maximum (60 cm) during cut-4, which was the physiologically mature stage of the crop growth.

Table I. Plant height (cm) of barseem fodder in response to various treatments at different cuts

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st cut</th>
<th>2nd cut</th>
<th>3rd cut</th>
<th>4th cut</th>
<th>5th cut</th>
<th>Treatment mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.0</td>
<td>48.0</td>
<td>55.0</td>
<td>54.7</td>
<td>63.3</td>
<td>51.9</td>
</tr>
<tr>
<td>Scarified seed</td>
<td>29.7</td>
<td>46.3</td>
<td>49.3</td>
<td>53.7</td>
<td>51.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Phosphorus fertilization</td>
<td>23.3</td>
<td>48.0</td>
<td>52.7</td>
<td>64.7</td>
<td>53.3</td>
<td>48.1</td>
</tr>
<tr>
<td>Rhizobia-inoculation</td>
<td>31.0</td>
<td>45.3</td>
<td>50.0</td>
<td>59.3</td>
<td>49.3</td>
<td>45.8</td>
</tr>
<tr>
<td>P-fertilization and Rhizobia-inoculation</td>
<td>29.5</td>
<td>48.0</td>
<td>54.0</td>
<td>67.5</td>
<td>55.0</td>
<td>48.7</td>
</tr>
<tr>
<td>Mean</td>
<td>30.1</td>
<td>47.1</td>
<td>52.1</td>
<td>60.0</td>
<td>54.3</td>
<td></td>
</tr>
</tbody>
</table>

Means in the same column with different superscripts are significantly different (P < 0.05)

Physiological efficiency of nitrogen (gm DM accumulated by unit of added nitrogen fixed in plant) was calculated according to Mengel and Kikiby (1989). The results showed that efficiency were not significantly affected by treatments and were 19.67, 25.33, 27.10 and 34 % with treatment B, C, D and E, respectively. These observations suggest that carbon assimilated could not keep pace with N absorption. Shaheen and RahmatUllah (1994) suggest that vegetation growth should be improved to obtain high physiological efficiency of nitrogen. In the present study unimproved seed was used purchased from local market, which due to limited growth potential may have constrained the physiological efficiency of nitrogen in the fodder.

The low mean phosphorus content in the fodder (0.21 %) may be attributed to the low pre-trial soil phosphorus concentration (71 mg kg \(-1\)) which agrees with the findings of Whiteman (1980) or the low availability as declined by alkaline calcareous nature of soil in Pakistan where the recovery is about 15-20% (Zia et al. 1991) and is one major limiting factor in crop yield (Ahmad et al. 1992). Anyhow post harvest phosphorus content in the soil increased in plots, which were fertilized with phosphorus.

Table II. Soil composition and root development characteristics of barseem

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil-N %</th>
<th>Soil-P %</th>
<th>Root Length (cm)</th>
<th>No. of Nodules/Plant</th>
<th>Root Hairs/Plant Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.10(^a)</td>
<td>58</td>
<td>5.4</td>
<td>137</td>
<td>++</td>
</tr>
<tr>
<td>Scarified seed</td>
<td>0.09(^ab)</td>
<td>60</td>
<td>4.5</td>
<td>112</td>
<td>+</td>
</tr>
<tr>
<td>Phosphorus fertilization</td>
<td>0.08(^ab)</td>
<td>80</td>
<td>6.0</td>
<td>146</td>
<td>++++</td>
</tr>
<tr>
<td>Rhizobia-inoculation</td>
<td>0.06(^b)</td>
<td>65</td>
<td>5.8</td>
<td>136</td>
<td>++++</td>
</tr>
<tr>
<td>P-fertilization and Rhizobia-inoculation</td>
<td>0.10(^a)</td>
<td>84</td>
<td>6.7</td>
<td>158</td>
<td>++++</td>
</tr>
</tbody>
</table>

Means in the same column with different superscripts are significantly different (P < 0.05)

(Root development; +: Poor, ++: Fair, +++: Developed, ++++: Well Developed)

Table II presents pot-harvest nitrogen and phosphorus contents of soil and root development parameters. Pre-trial soil nitrogen content was 0.12%, which apparently did not change in response to any treatment. Mean soil-N after the last cutting of barseem was 0.068% across the five treatments. This is in contrast to the findings of Asad (1978), who reported an increase in soil nitrogen concentration in response to rhizobium inoculation of barseem. Failure to find any increase in soil-N in the present study was presumably due to the reasons that soil was not irrigated and ploughed before sampling, which did not release the withhold nitrogen in the barseem roots. Nevertheless it has been suggested that legume contribute nitrogen only to that fraction of soil-N pool which is available to the plant (Shah, personal communication). Such added nitrogen if determined separately may constitute a significant entity. Moreover, N-contents of soil did not show any predictable trend, as rhizobium activity was expected to enhance. However, the soil contains its own harboring rhizobia, which may have performance comparable to those introduced.

Phosphorus application and rhizobium inoculation in isolation or in combination tended to enhance root development i.e. root length, number of nodules and root hairs per plant. It was the large variation between the replicates with in the treatment, which made the effect on root development statistically insignificant (Table II). Mashiatullah et al., (1987) and Shaheen and RahmatUllah (1994) reported an increase in root length and nodules
number per plant with the application of P-fertilizer and rhizobium inoculation. It is assumed that lack of significant response to root development for different treatments may be attributed to comparatively low appropriateness of rhizobium used in the present study and/or deficiency of co-factors such as trace minerals in the soil (Khan et al. 1987). However, the average number of nodules per plant (138) in the present study was markedly higher than the maximum number (71) reported by Khan et al., (1987) for local varieties of barseem.

It is concluded that plant height was significantly affected by the treatments, particularly during the physiological mature stage of growth. Total-N and total phosphorus content in the soil was not affected by either treatment under conditions of the present study. Although root length and nodules count were not significantly affected, but the effect of treatments was quite visible on root branching. It is suggested that improved variety of seeds of high potential yield should be used to exploit full benefit from agronomic innovations. Appropriate strain of rhizobium should be used to maximize barseem yield and soil fertility. This would need research to evaluate the efficiency of different rhizobia under variable soil conditions and seed types.

CONCLUSION AND RECOMMENDATIONS

Scarification did not affect the plant height, soil N or P contents. However, P-fertilization added in building up soil-phosphorus reserves for the following crop. Post-harvest phosphorus content in the soil increased in plots, which were fertilized with phosphorus. Phosphorus application and rhizobium inoculation in isolation or in combination tended to enhance root development i.e., root length, number of nodules, root branching and root hairs per plant. Phosphorus fertilization in isolation or in combination with rhizobium inoculation enhanced phosphorus reserves of the soil and made the root vigorous. The indigenous rhizobium may have comparable performance and could not contribute to N-reserves of the post-harvest soil.

REFERENCES


Sohail Ahmad et al. Effect of seed scarification, rhizobium inoculation and phosphorus fertilization root… 374